

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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| Applicant: | Frank Luyten | Art Unit: | 1655 |
| Serial No.: | 10/089,932 | Examiner: | A.P. Wood |
| Filed: | July 29, 2002 | Customer No.: | 21559 |
| Title: | IN VIVO ASSAY AND MOLECULAR MARKERS FOR TESTING THE PHENOTYPIC STABILITY OF CELL POPULATIONS AND SELECTED CELL POPULATIONS FOR AUTOLOGOUS TRANSPLANTATION | | |

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

REPLY TO RESTRICTION REQUIREMENT

In reply to the Restriction Requirement that was mailed in connection with the above-captioned application on January 4, 2006, and prior to examination of the application, Applicants submit the following Amendment and Remarks.

Amendments to the claims:

1-28 (cancelled)

29. (withdrawn) An assay to identify molecular markers linked to phenotypic stability of a chondrocyte cell population comprising:

- a) providing a suspension of isolated or expanded cells and determining positive and negative markers thereof,
- b) injecting intramuscularly or subcutaneously in a non-human animal said suspension of isolated or expanded cells in an iso-osmotic liquid, the same suspension comprising articular chondrocytes in an amount equivalent to at least 1×10^6 chondrocytes as applied to immune-deficient mice,
- c) allowing the formation of cartilaginous tissue,
- d) sacrificing the animal,
- e) evaluating the in vivo formed cartilage histologically for stable, non-vascularised cartilage, and
- f) identifying positive or negative molecular markers of those isolated or expanded cells which formed stable, non-vascularised cartilage in vivo, as evaluated in step e).

30. (withdrawn) An assay to identify molecular markers according to claim 29, comprising using freshly isolated or serially passaged cells and using differential gene expression analysis methods selected from the group consisting of differential display, subtractive hybridization, subtracted libraries or cDNA chips and cDNA arrays to identify said positive or negative molecular markers of those isolated or expanded cells which formed stable, non-vascularized cartilage in vivo.

31. (currently amended) A method to identify cells having chondrocyte phenotypic stability comprising determining the expression of a positive marker of chondrocyte phenotypic stability which is BMP-2 and/or FGFR-3 and/or a markers co-detectable with these markers identified identifiable by the a method of claim 29- comprising the steps of:

- a) providing a suspension of isolated or expanded cells and determining a

positive marker thereof,

- b) injecting intramuscularly or subcutaneously in a non-human animal said suspension of isolated or expanded cells in an iso-osmotic liquid, the same suspension comprising articular chondrocytes in an amount equivalent to at least 1×10^6 chondrocytes as applied to immune-deficient mice,
- c) allowing the formation of cartilaginous tissue,
- d) sacrificing the animal,
- e) evaluating the in vivo formed cartilage histologically for stable, non-vascularised cartilage, and
- f) identifying a positive molecular marker of those isolated or expanded cells which formed stable, non-vascularised cartilage in vivo, as evaluated in step e) and/or specific reporter constructs comprising a promoter of said markers.

32. (currently amended) A method to identify cells having chondrocyte phenotypic stability according to claim 31 further comprising determining that activin-like kinase-1 (ALK-1) is not expressed, and/or a markers is not expressed which is a marker co-detectable with these said ALK-1 markers identified by the a method of claim 29 comprising the steps of:

- a) providing a suspension of isolated or expanded cells and determining a negative marker thereof,
- b) injecting intramuscularly or subcutaneously in a non-human animal said suspension of isolated or expanded cells in an iso-osmotic liquid, the same suspension comprising articular chondrocytes in an amount equivalent to at least 1×10^6 chondrocytes as applied to immune-deficient mice,
- c) allowing the formation of cartilaginous tissue,
- d) sacrificing the animal,
- e) evaluating the in vivo formed cartilage histologically for stable, non-vascularised cartilage, and
- f) identifying a negative molecular marker of those isolated or expanded cells which formed stable, non-vascularised cartilage in vivo, as evaluated in step e)
and/or specific reporter constructs comprising a promoter of said markers are not expressed.

33. (withdrawn - currently amended) A~~The~~ method to identify cells with chondrocyte phenotypic stability according to claim 31, comprising

- a) ~~hybridising sets of DNA probes to messenger RNA from cells, sets of DNA probes provided on DNA arrays or DNA chips, said DNA probes being probes of said positive and negative markers for chondrocyte phenotypic stability BMP-2 and/or said FGFR-3 and/or said markers co-detectable with these markers; and~~
- b) Identifying those cells which (i) hybridise with said probes of said positive markers and (ii) ~~do not hybridise with said negative markers for chondrocyte phenotypic stability.~~

34. (withdrawn - currently amended) A~~The~~ method to identify phenotypically stable ~~primary chondrocytes and chondrocytes~~ according to claim 31, said method comprising detecting said set of positive markers in a cells from a cartilage biopsy, after at least one passage, ~~that remained phenotypically stable comprising detecting sets of positive markers, said positive markers being selected from expressed BMP-2, FGFR-3, markers co-detectable with these markers identifiable by the method of claim 29 or specific reporter constructs comprising a promoter of said markers.~~

35. (withdrawn - currently amended) The method to identify phenotypically stable chondrocytes according to claim 31, which method is used ~~Method~~ to monitor passage by passage cell expansion and/or to predict when cell expansion must be stopped and/or to recover cells that have already lost their phenotypic stability only when needed and/or to provide a means for quality control of cells to be used for autologous cell transplantation and/or selecting from a cell population only those cells that retain their chondrocyte phenotypic stability;

~~comprising detecting the expression of molecular markers of chondrocyte phenotypic stability selected from the group of:~~

- ~~(i) markers determined by the assay comprising: a) injecting intramuscularly or subcutaneously in a non-human animal a suspension of isolated or expanded cells in an iso-osmotic liquid, the same suspension comprising articular chondrocytes in an amount equivalent to at least 1×10^6 chondrocytes as applied to immune deficient mice, b) allowing the formation of cartilaginous tissue, c) sacrificing the animal, d) evaluating the formed cartilage histologically for stable, non-vascularised cartilage in~~

~~vivo, and e) identifying positive or negative molecular markers of those isolated or expanded cells evaluated in step d) which form stable, non-vascularised cartilage in vivo.~~

~~(ii) BMP-2 and/or FGFR-3 and/or markers co-detectable with these markers identifiable by the method of claim 29 and/or specific reporter constructs associated with these markers.~~

~~(iii) expressed activin-like kinase-1 (ALK-1) as a marker negatively associated with chondrocyte phenotypic stability, and/or markers co-detectable with these markers identifiable by the method of claim 29 and/or specific reporter constructs comprising a promoter of said markers.~~

36. (withdrawn - currently amended) Method according to claim 35, comprising sorting said phenotypically stable chondrocytes cells via monoclonal or polyclonal antibodies against ~~negative or~~said positive markers or said co detectable markers, ~~for the monitoring of cell expansion and/or predicting when cell expansion must be stopped and/or selecting cells which have lost chondrocyte phenotypic stability and/or selecting cells which retain chondrocyte phenotypic stability.~~

37. (cancelled)

38. (cancelled)

39. (withdrawn) Cells identified according to a method comprising:

(a) hybridising sets of DNA probes to messenger RNA from cells, said DNA probes being probes of positive and negative markers for chondrocyte phenotypic stability; and

(b) identifying those cells that (i) hybridise with said positive markers and (ii) do not hybridise with said negative markers for chondrocyte phenotypic stability.

40. (withdrawn) Cells selected according to a method comprising

(a) detecting the expression of molecular markers of chondrocyte phenotypic stability selected from the group consisting of :

(i) BMP-2 and/or FGFR-3 and/or markers co-detectable with these markers

identified by the method of claim 29 and/or specific reporter constructs associated with these markers, which markers are positive markers for chondrocyte phenotypic stability, and

(ii) expressed activin-like kinase-1 (ALK-1) as a marker negatively associated with chondrocyte phenotypic stability, and/or markers co-detectable with these markers identified by the method of claim 29 and/or specific reporter constructs comprising a said marker; and

(b) selecting cells in which expression of positive markers and absence of markers negatively associated with phenotypic stability is detected.

41. (withdrawn) An assay to predict the outcome of autologous cell transplantation comprising

(a) providing isolated or expanded chondrocytes;

(b) subcutaneously or intramuscularly injecting in a non-human animal of a suspension of the selected cells in an iso-osmotic liquid, said suspension comprising articular chondrocytes in an amount equivalent to at least 1×10^6 chondrocytes as applied to immune-deficient mice;-

(c) allowing the formation of cartilaginous tissue;

(d) sacrificing the animal; and

(e) evaluating the in vivo formed cartilage histologically for stable, non-vascularised cartilage; whereby the formation of stable, non-vascularised cartilage is indicative of a positive outcome of autologous cell transplantation using said isolated or expanded chondrocytes.

42. (cancelled)

43. (previously presented) A method of transplanting cells to a connective tissue site in a patient or a method of seeding with cells any prosthetic device intended to be anchored into a mammal, wherein said cells are cells which retain their chondrocyte phenotypic stability and which are identified according to the method of any one of claims 31 to 33.

44. (withdrawn – currently amended) A method of transplanting cells to a connective tissue site in a patient or a method of seeding with cells any prosthetic device intended to be anchored into a mammal, wherein said cells are cells which retain their chondrocyte phenotypic stability and which are selected according to the method of claims 35.

45. (previously presented) A therapeutic composition for humans including cells identified according to claim any one of claims 31 to 33, optionally further including at least a pharmaceutically acceptable carrier and/or a growth factor.

46. (withdrawn) A therapeutic composition for humans including cells selected according to claim 35, optionally further including at least a pharmaceutically acceptable carrier and/or a growth factor.

47. (withdrawn) A diagnostic comprising positive or negative markers identified according to the method of claim 29.

48. (cancelled)

49. (withdrawn) A cell culture exhibiting chondrocyte phenotypic stability in which the cells express a ratio of BMP-2 and/or FGFR-3 as molecular markers positively associated with chondrocyte phenotypic stability and/or markers co-detectable with these markers identified by the method of claim 29 and/or specific reporter constructs comprising associated with these markers to activin-like kinase-1 (ALK-1) as a molecular marker negatively associated with chondrocyte phenotypic stability and/or markers co-detectable with this marker identified by the method of claim 29 and/or specific reporter constructs comprising a promoter of said negative marker, which is greater than 1.

50. (withdrawn) A cell culture exhibiting chondrocyte phenotypic stability in which culture does not express activin-like kinase-1 (ALK-1) and/or markers co-detectable with this marker identified by the method of claim 29 and/or specific reporter constructs comprising a promoter of said markers.

51. (previously presented) The method of claim 33, wherein said DNA probes are provided on a DNA array or a DNA chip.

52. (withdrawn) The method of claim 39, wherein said DNA probes are provided on a DNA array or a DNA chip.

53. (withdrawn) The diagnostic of claim 47, wherein said positive or negative markers are DNA probes.

54. (withdrawn) The cell culture exhibiting chondrocyte phenotypic stability, according to claim 49, wherein said ratio is greater than 2.

55. (new) The method to identify cells with chondrocyte phenotypic stability according to claim 32, comprising

a.) hybridising to messenger RNA from cells, sets of DNA probes provided on DNA arrays or DNA chips, said DNA probes being probes of said negative marker for chondrocyte phenotypic stability ALK-1 and/or said marker co-detectable with ALK-1; and

b) identifying those cells which hybridise with said probe of said positive marker for chondrocyte phenotypic stability.

56. (new) The method to identify cells having chondrocyte phenotypic stability of claim 31, said method comprising determining the expression of a positive marker of chondrocyte phenotypic stability which is BMP-2.

57. (new) The method to identify cells having chondrocyte phenotypic stability of claim 31, said method comprising determining the expression of a positive marker of chondrocyte phenotypic stability which is FGFR-3.

58. (new) The method to identify cells having chondrocyte phenotypic stability of claim 33, wherein said positive marker of chondrocyte phenotypic stability is BMP-2.

59. (new) The method to identify cells having chondrocyte phenotypic stability of claim 33, wherein said positive marker of chondrocyte phenotypic stability is FGFR-3.

REMARKS

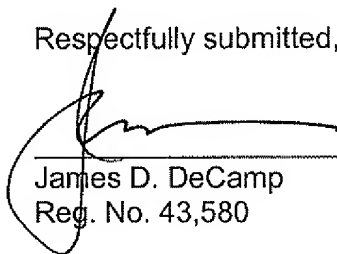
In reply to the Restriction Requirement that was mailed in connection with the above-captioned case on January 4, 2006 applicant elects the invention of Group II, claims 31-32, 43, and 45. The election is made without traverse.

In addition, applicants respectfully request that, in view of the present amendment, claims 33, 34, 35, 36, 44, and 51 be rejoined to Group I. Claims 33-35 have been amended to depend from elected claim 31. Claims 36 and 44 have been amended to depend from claim 35 which, in turn, depends from elected claim 31. Claim 51 now depends, through claim 35, on claim 31. New claims 55-59 have also been added. And elected claims 31 and 32 have been amended to incorporate subject matter of claim 29. Support for each of these amendments is found in the application as filed. No new matter is added by these amendments.

If there are any charges or any credits, please apply them to Deposit Account No. 03-2095.

Respectfully submitted,

Date: 6 February 2006



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